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# Preclinical Studies on LY228729: A Potent and Selective Serotonin$_{1A}$ Agonist

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## ABSTRACT

LY228729 is a conformationally restricted tryptamine derivative with a carboxamide serving as a protophilic group to mimic the hydroxyl in serotonin (5-HT). LY228729 has high affinity for the 5-HT$_{1A}$ receptor, weak affinity for the 5-HT$_{1D}$ receptor and no significant affinity for other monoaminergic receptors studied. LY228729 was less effective than 5-carboxamidotrytamine in suppressing $K^+$-evoked release of $^3$H-5-HT from parietal-occipital cortical slices from guinea pigs, which is in agreement with its weak 5-HT$_{1D}$ receptor affinity. LY228729 reduced hypothalamic 5-hydroxyindole-3-acetic acid levels and increased serum corticosterone levels in rats. LY228729 reduced hypothalamic 5-hydroxytryptophan accumulation after decarboxylase inhibition. LY228729 increased flat posture and lower lip retraction scores in rats at doses between 0.1 and 1 mg/kg s.c. (p.o. doses were 10 times higher) and these effects were blocked by (±) pindolol. LY228729 induced a hypothermic response in rats, which was blocked by (±) pindolol. These *in vivo* responses are characteristics of compounds with 5-HT$_{1A}$ agonist activity. In the preclinical efficacy models, LY228729 suppressed motion sickness responses in cats; decreased ejaculatory latency and the increased copulatory efficiency and rate in rats and increased punished responding at lower doses than it lowered unpunished responding in rats. Collectively, these results indicate that LY228729 is potent 5-HT$_{1A}$ agonist with bioavailability properties sufficient for clinical evaluation and with efficacy in preclinical models of anxiety, sexual disorders and motion sickness. Since the 5-HT$_{1A}$ agonists that have been studied previously have antidepressant activity, this indication will also be evaluated.

The 5-HT receptors are currently divided into the 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptor families (Peroutka *et al.*, 1990; Bockaert *et al.*, 1992). The 5-HT$_1$ family consists of the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptor subtypes (Peroutka *et al.*, 1990; Leonhardt *et al.*, 1989; Zgombick *et al.*, 1992; Adham *et al.*, 1993). Among these receptors, the 5-HT$_{1A}$ receptor has been extensively studied due to the availability of selective ligands, such as 8-OH-DPAT and clinically effective compounds with 5-HT$_{1A}$ receptor activity, such as buspirone, ipsapirone, gepirone and flesinoxan. These ligands provided a means to identify 5-HT$_{1A}$ receptor-mediated responses and to evaluate the role of these receptors in preclinical models that are considered to be predictive of efficacy in clinical pathologies. In male rats, the effects of 8-OH-DPAT that are characteristic of 5-HT$_{1A}$ receptor activation include presynaptic responses, such as reductions in the synthesis, utilization and turnover of 5-HT (Hjorth and Magnusson, 1988) and suppression of firing of 5-HT neurons (Sprouse and Aghajanian, 1986) and putative postsynaptic responses, such as reductions in body temperature (Hjorth, 1985), stimulation of adrenocorticotropin hormone, corticosterone and β-endorphin secretion (Koenig *et al.*, 1987; Gilbert *et al.*, 1988), and induction of stereotypic behaviors, such as lower lip retraction (Berendsen *et al.*, 1989), flat posture, hind limb abduction and forepaw treading (Smith and Peroutka, 1986). The proposed clinical utilities for 5-HT$_{1A}$ receptor agonists include the treatment of anxiety, depression, motion sickness and various types of sexual disorders (Taylor, 1990; Foreman and Wernicke, 1990). The justification for these utilities stem from preclinical studies and clinical trials that evaluated the effects of the partial 5-HT$_{1A}$ agonists buspirone, gepirone and ipsapirone (Othmer and Othmer, 1987; Taylor, 1990) and the responses of other 5-HT$_{1A}$ receptor agonists in preclinical models of anxiolytic activity (Barrett and Gleeson, 1991), antidepressant activity (Wieland and Lucki, 1990) and sexual response (Ahlenius *et al.*, 1981; Glaser *et al.*, 1987).

To improve on the clinical efficacy of these compounds, medicinal chemistry efforts have focused on the development of an agonist for the 5-HT$_{1A}$ receptor with high potency, selectivity and suitable bioavailability. One chemical series that has been developed with these properties is the 1,3,4,5-tetrahydrobenz[cd]indol-4-amines with protophilic substitutions in the 6-position (Mason *et al.*, 1987; Glaser *et al.*, 1987; Flaugh *et al.*, 1988; Slaughter *et al.*, 1990). These compounds are conforma-

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ABBREVIATIONS: 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HTP, 5-hydroxytryptophan; 5-CT, 5-carboxamidotryptamine; 5-HT, serotonin; GABA, *gamma*-aminobutyric acid; NSD 1015, 3-hydroxybenzylhydrozine dichloride.

tionally restricted tryptamines or ABC partial ergolines (Bach *et al.*, 1980) with protophilic groups at the analogous position that is occupied by the hydroxyl group in 5-HT (Flaugh *et al.*, 1988). Among these compounds, LY228729 (fig. 1) had the highest potency, efficacy, oral activity and selectivity for the 5-HT$_{1A}$ receptor (Mason *et al.*, 1987; Flaugh *et al.*, 1988; Slaughter *et al.*, 1990). The present report summarizes the preclinical pharmacological studies with LY228729 that evaluated the binding affinity in a variety of radioligand displacement assays, *in vivo* serotonergic activity and pharmacokinetic profile.

## Methods

**Materials.** LY228729 [(−)-4-(dipropylamino)-1,3,4,5-tetrahydrobenz-[c,d,]indole-6-carboxamide], 6-$^{14}$C-LY228729, LY228730 [(+)-4-(dipropylamino)-1,3,4,5-tetrahydrobenz[c,d]indole-6-carboxamide], LY289211 [(−)-4-(propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole-6-carboxamide] and (−)-4-dipropylamino-1,3,4,5-tetrahydro-4-propylaminobenz[c,d]indole-6-carbonitrile were synthesized in the Lilly Research Laboratories (Indianapolis, IN; Flaugh *et al.*, 1988; Martinelli *et al.*, 1990; Swanson and Catlow, 1992). 8-OH-DPAT, methiothepin and 5-CT were purchased from Research Biochemicals (Natick, MA). NSD 1015 was purchased from Aldrich Chemical Company (Milwaukee, WI). All other radioactive isotopes used in these studies were obtained from New England Nuclear (Boston, MA).

**Radioligand displacement assays.** The binding affinities for LY228729 and LY228730 were evaluated using ligand displacement assays for the following receptors: *alpha*$_1$ adrenergic, *alpha*$_2$ adrenergic, *beta* adrenergic, $D_1$ dopaminergic, $D_2$ dopaminergic, benzodiazepine, $H_1$ histaminergic, GABA-A, muscarinic, 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, 5-HT$_2$ and 5-HT$_3$. The general assay conditions are defined in table 1. The benzodiazepine, $H_1$ histaminergic, *alpha*$_2$, *alpha*$_1$, *beta* adrenergic receptor assays utilized frozen rat whole brain tissue; the muscarinic and GABA-A receptor assays utilized frozen rat cerebral cortex. The dopaminergic assays utilized frozen rat corpus striatum. All frozen tissues were obtained from Pel Freez Biologicals (Rogers, AR). The 5-HT$_{1A}$ assays utilized fresh or frozen (−70°C) hippocampal tissues obtained from male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI). The 5-HT$_{1B}$, 5-HT$_2$ and 5-HT$_3$ assays utilized fresh cerebral cortical tissues obtained from male Sprague-Dawley rats. The 5-HT$_{1C}$ assays utilized fresh choroid plexus tissues obtained from bovine brains immediately after slaughter. The 5-HT$_{1D}$ assays utilized caudate tissues obtained from fresh bovine brains purchased from Pel Freez Biologicals and frozen (−70°C) until the day of the assay. The other reagents and conditions used in each assay are described in table 1.

The frozen rat tissues used for the nonserotonergic receptor assays were homogenized in 30 volumes of buffer (defined by references in table 1) using a Brinkmann Polytron (Westbury, NY) at a (setting of 6 for 15 sec ×2). The homogenates were centrifuged at 1000 × *g* for 10 min and the resultant fraction was centrifuged at 40,000 × *g* for 10 min. Cortical membranes that were used for the GABA-A receptor assay were also subjected to freeze-thawing and solutions containing Triton X-100 for removal of endogenous GABA (Williams and Risley, 1979).

The rat hippocampal membranes used for 5-HT$_{1A}$ assays were prepared by homogenizing the tissue in 40 volumes of ice-cold Tris HCl buffer (50 mM, pH 7.4) using a Teckmar Tissumizer (Cincinnati, Ohio) at a setting of 65 for 15 sec and the homogenate was centrifuged for 10 min at 39,800 × *g*. The resulting pellet was then resuspended in the same buffer and the centrifugation and resuspension process was repeated three times to wash the membranes. Between the second and third washes, the resuspended membranes were incubated for 10 min at 37°C to facilitate the removal of endogenous ligands. The final pellet was resuspended in 67 mM Tris HCl, pH 7.4, to a concentration of 2 mg of tissue original wet weight per 200 μl. This homogenate was frozen (−70°C) until the day of the assay.

The bovine caudate tissues used for the 5-HT$_{1D}$ assays were homogenized and processed identically to the membranes used for the 5-HT$_{1A}$ assays. The final pellet was resuspended in 67 mM Tris HCl, pH 7.4, to a concentration of 25 mg of original tissue wet weight per milliliter. The 5-HT$_{1B}$ assays utilized fresh cerebral cortical tissues obtained from male Sprague-Dawley rats (Harlan Industries, Cumberland, IN).

The bovine choroid plexus tissues used for the 5-HT$_{1C}$ assays were homogenized in nine volumes of 0.32 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 17,000 × *g* for 20 min and the resulting pellet was suspended in 100 volumes of Tris HCl buffer (50 mM, pH 7.4). This suspension was incubated for 10 min at 37°C and centrifuged at 50,000 × *g* for 10 min. The resuspension, incubation and centrifugation processes were repeated three times and the final pellets were frozen (−70° C) and used within 2 weeks according to the method of Hoyer and Karpf (1988).

The rat cerebral cortical tissues used for the 5-HT$_{1B}$, 5-HT$_2$ and 5-HT$_3$ assays were homogenized in nine volumes of 0.32 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 17,000 × *g* for 20 min. The resulting pellet was resuspended in 100 volumes of 50 mM Tris HCl (pH 7.4), incubated for 10 min at 37°C and centrifuged at 50,000 × *g* for 10 min. The pellets were resuspended and centrifuged three additional time. The final pellets were frozen at −70°C and used within 2 weeks.

**Effects on K$^+$-evoked $^3$H-5-HT release from slices of guinea pig parietal-occipital cortex.** Male guinea pigs weighing 150 to 160 g were purchased from Charles River Breeding Laboratories. The animals were sacrificed by decapitation; the brains were rapidly removed and the parietal-occipital cortex was dissected. This cortical tissue was divided into 3-mm strips and sliced into sections 0.3-mm thick using a McIlwain tissue chopper. These sections were incubated for 30 min at 37°C in a buffer containing 118 mM NaCl, 25 mM $NaHCO_3$, 11.1 mM β-D(+)glucose, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, 0.57 mM L-ascorbic acid, 0.03 mM $Na_2$-EDTA, 0.01 mM pargyline and 100 nM 5[1,2 $^3$H]-HT creatine sulfate (25 Ci/mmol, New England Nuclear, Wilmington, DE), which had been gassed with 95% $O_2$/5% $CO_2$. Following this labeling procedure, the buffer was removed and the slices were washed with the same buffer without isotope. The slices were transferred to 100-μl superfusion chambers and superfused at a rate of 1 ml/min. The basal release buffer was the same as the wash buffer except that it contained 3.0 μM fluoxetine. The stimulus buffer was identical to this buffer except it had the NaCl and KCl concentrations adjusted to 92.8 and 30 mM, respectively. The tissues were exposed to the following sequence of buffers: basal buffer without drug for 35 min ($SP_1$), stimulus buffer without drug for 3 min ($S_1$), basal buffer without drug for 17 min, basal buffer with drug for 15 min ($SP_2$), stimulus buffer with drug for 3 min ($S_2$) and basal buffer with drug for 17 min. In additional experiments metitepine ($10^{-6}$ M) was used to antagonize the effects of LY228729 ($10^{-6}$ M). This concentration of metitepine was chosen because it did not induce significant increases in basal or stimulus-evoked release of tritium under the conditions used in this experiment. Effluent from the chambers was collected at 5-min intervals and the tissues with chamber contents were removed at the end of the experiments. These samples were combined with 12 ml of scintillation cocktail (Ready Solv HP, Beckman Instruments, Fullerton, CA) and the radioactivity was estimated by liquid scintillation spectrometry. The effects of test compounds on the release of 5-HT were estimated by calculating the

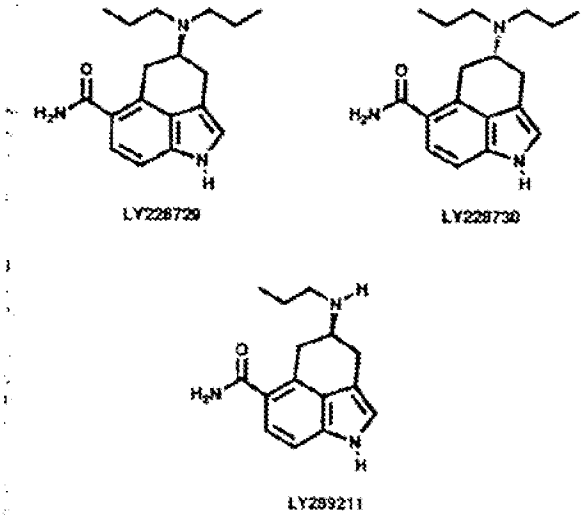


Fig. 1. Structures of LY228729, its opposite enantiomer (LY228730) and its despropyl metabolite (LY289211).

TABLE 1

Assay conditions for $^3$H-ligand displacement studies

| Receptor | $^3$H-Ligand (nM) | Buffer* | Nonspecific Ligand (μM) | Minutes (°C) | Reference |
|---|---|---|---|---|---|
| 5-$HT_{1A}$ | 8-OH-DPAT (1) | A | 5-HT (10) | 15/37 | Taylor *et al.*, 1986 |
| 5-$HT_{1B}$ | 5-HT (2) | B | 5-HT (10) | 30/22 | Wong *et al.*, 1991 |
| 5-$HT_{1C}$ | Mesulergine (1) | C | 5-HT (10) | 30/37 | Pazos *et al.*, 1984 |
| 5-$HT_{1D}$ | 5-HT (2) | D | 5-HT (10) | 10/37 | Xiong and Nelson, 1989 |
| 5-$HT_2$ | Ketanserin (0.5) | E | Methysergide (10) | 15/37 | Taylor *et al.*, 1986 |
| 5-$HT_3$ | LY278584 (0.5) | F | 5-HT (10) | 30/25 | Wong *et al.*, 1989 |
| GABA-A | Muscimol (2) | G | GABA (10) | 60/4 | Williams and Risley, 1979 |
| Benzodiazepine | Flunitrazepam (2) | G | Clonazepam (10) | 60/4 | Braestrup and Squires, 1978 |
| $D_1$ | SCH23390 (0.2) | H | SCH23390 (0.03) | 60/23 | Seeman *et al.*, 1979 |
| $D_2$ | Raclopride (0.8) | H | Spiperone (0.03) | 60/23 | Creese and Snyder, 1978 |
| ACh-M | QNB (1) | I | Atropine (1.0) | 60/23 | Yamamura and Snyder, 1974 |
| $H_1$ | Pyrilamine (2) | J | Promethazine (10) | 30/23 | Tran *et al.*, 1978 |
| *Alpha*$_1$ adrenergic | Prazosin (0.2) | K | WB4101 (0.1) | 60/23 | Greengrass and Bremner, 1979 |
| *Alpha*$_2$ adrenergic | Clonidine (2.0) | L | Mianserin (10) | 30/23 | Braunwalder *et al.*, 1981 |
| *Beta* adrenergic | DHA (0.2) | K | (−) Propranolol (1.0) | 60/23 | Bylund and Snyder, 1976 |

* A = 50 mM Tris HCl, 3 mM $CaCl_2$, 0.01 mM pargyline, pH 7.4; B = buffer A + 100 nM SCH23390; C = buffer A + 5.7 mM ascorbate, 30 nM spiperone, pH 7.4; D = buffer A + 5.7 mM ascorbate, 100 nM 8-OH-DPAT, 100 mM mesulergine, pH 7.4; E = 50 mM Tris HCl, pH 7.6; F = 50 mM Tris HCl, 5 mM $CaCl_2$, 0.01 mM pargyline, 0.6 mM ascorbate, pH 7.4; G = 50 mM Tris HCl, pH 7.4; H = buffer F + 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$; I = 50 mM $Na_2HPO_4$, pH 7.4; J = 50 mM $Na_2HPO_4$, pH 7.5; K = 50 mM Tris HCl, pH 7.7; L = buffer J + 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$.

fractional release at each collection interval and the ratio of the stimulus-evoked overflow with test compound ($S_2$) and without test compound ($S_1$) according to the methods described previously (Engel *et al.*, 1983). In these experiments, the $SP_1$, $SP_2$, $S_1$, $S_2$, and $S_2/S_1$ values for control chambers ($n$ = 37) were 4.93 ± 0.12%, 2.72 ± 0.10%, 2.43 ± 0.18%, 3.35 ± 0.24% and 1.40 ± 0.04. The tissue dpm remaining at the end of the experiment was 88,009 ± 4088. Statistical comparisons were made using a one-way analysis of variance followed by a Dunnett's test with the minimal level of significance of $P < .05$.

**Evaluation of changes in concentrations of monoamines and their metabolites in rat brains.** Male Sprague-Dawley rats weighing 170 to 200 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN). At specified times after the compound administration, the rats were decapitated and the brain areas were dissected and frozen on dry ice. The tissues were stored at −15°C until analysis. On the day of analysis, the tissues were sonicated in 10 volumes of 0.1 M trichloroacetic acid containing 0.1 mg/ml cysteine as a stabilizing agent and an internal standard (described subsequently). The homogenates were centrifuged at 12,000 × *g* for 5 min and the supernatants were analyzed by two high-pressure liquid chromatography methods with electrochemical detection for either 5-HT and 5-HIAA or 5-HTP. In the assay for 5-HIAA and 5-HT, 30 μl of the supernatants were injected onto an Econosphere C18 column (5μ, 4.6 × 150 mm) purchased from Alltech (State College, PA) and chromatographed using 40 ng/ml 5-hydroxyindole-3-carboxylic acid as an internal standard and a mobile phase consisting of 0.1 M monochloracetic acid, 1 mM EDTA, 220 mg/liter sodium octyl sulfate, 8% acetonitrile, pH 2.6, at a flow rate of 1 ml/min and a temperature of 40°C. In the assay for 5-HTP, 30 μl of the supernatants were injected onto a C-18 column (described previously) and chromatographed using an internal standard of 40 ng/ml α-methyldopa and a mobile phase consisting of 0.1 M monochloracetic acid, 1 mM EDTA, 50 mg/liter sodium octyl sulfate, 5% methanol, pH 2.6, a flow rate of 1 ml/min and a temperature of 40°C. The instrumentation consisted of a BAS LCEC Analyzer (Bioanalytical Systems, West Lafayette, IN) and a Waters (Itasca, IL) WISP automatic sample injector. The BAS glassy carbon electrode was used at a potential of +0.75 V for all assays. Statistical evaluations were made using a Student's $t$ test and the minimal level of significance was $P < .05$.

**Determination of serum corticosterone concentrations in rats.** Male Sprague-Dawley rats (Harlan Sprague-Dawley) were used in these studies. At 1 hr after compound administration, the rats were decapitated and trunk blood was collected and allowed to clot. Serum samples were obtained by centrifugation of the clotted blood and were stored frozen (−15°C) before analysis. Serum corticosterone concentrations were determined by the spectrofluorometric method of Solem and Brinck-Johnsen (1965). Statistical evaluations were made using a Student's $t$ test and the minimal level of significance was $P < .05$.

**Evaluation of 5-$HT_{1A}$ receptor-mediated behaviors in the rat.** Male Sprague-Dawley rats (5–6 months of age) were purchased from Charles River Breeding Laboratories. The 5-$HT_{1A}$ receptor-mediated behaviors recorded in these tests included lower lip retraction and flat posture. The lower lip retraction response was evaluated using the 0 to 1 scale with 0.5 representing a moderate response that has been previously established for 5-$HT_{1A}$ receptor agonists (Tricklebank *et al.*, 1984; Berendsen *et al.*, 1989). The flat posture response was evaluated using the 0 to 3 scale, which has also been previously established (Smith and Peroutka, 1986). The behavioral evaluations were made at 5-min intervals immediately after treatment and ending 90 min later. In separate experiments, the effects of pretreatment with 10 mg/kg s.c. (±) pindolol on the responses to LY228729 (0.3 mg/kg s.c.) were evaluated. The dose of pindolol was chosen because it did not induce significant behavioral responses when given alone. The treatment-induced effects were analyzed using the Mann-Whitney $U$ test (Zar, 1974).

**Effects on body temperature in rats.** Male, Sprague-Dawley rats (175–250 g) were purchased from Charles River Breeding Laboratories and were acclimated to the housing environment for at least 1 week before testing. Body temperature was measured with a Bailey Instruments Thermometer, Model Bat 8 (Saddle Brook, NJ) by lubricating the probe tip with corn oil and inserting it approximately 3 cm into the rectum for 10 to 15 sec. Measurements were taken just before compound administration and at 30 min after s.c. treatment. The changes in body temperature were evaluated by determining the differences temperatures after treatment compared with the pretreatment values. In separate experiments, 3 mg/kg s.c. (±) pindolol, a 5-$HT_{1A}$/*beta* adrenergic antagonist, was given 15 min before LY228729. The dose of pindolol was chosen because it did not induce changes in body temperature when given alone. The treatment-induced effects were analyzed using the Mann-Whitney $U$ test (Zar, 1974).

**Evaluation of male rat sexual behavior.** All rats used in these studies were housed in a temperature-controlled room in which the lights were off from 10:00 A.M. to 8:00 P.M.. Male Sprague-Dawley rats and ovariectomized Long-Evans rats purchased from Charles River Breeding Laboratories were used in these studies. The ovariectomized rats used as sexual partners for the test male rats were made sexually receptive by administering 400 μg of estrone in propylene glycol s.c. 48 hr before testing and 2.5 mg of progesterone in propylene glycol s.c. 4 hr before testing. The male rats were individually housed beginning 4 weeks before testing and were tested at 2-week intervals beginning at 6 months of age and ending at 12 months of age using the previously published procedure (Foreman and Hall, 1987, Foreman *et al.*, 1992). Mating tests were conducted between 12:00 noon and 5:00 P.M. during the dark phase of the lighting cycle. Each behavioral test was initiated with the introduction of a receptive female rat into the arena and was terminated either 30 min later or immediately after the first postejaculatory mount. Before treatment with a drug solution, each male rat was required to have at least two consecutive vehicle tests with similar sexual performance. After each drug testing, additional vehicle tests were performed. In an effort to eliminate behavioral responses with drug treatment that might be due to spontaneous changes in base-line

TABLE 2

**Affinity constants for LY228729 and LY228730**

Values are mean ± S.E.M. The minimal number of experiments for each mean was three.

| Receptor | Apparent $K_i$ (nM) | | |
|---|---|---|---|
| | LY228729 | LY228730 | LY288211 |
| 5-HT$_{1A}$ | 0.13 ± 0.01 | 0.28 ± 0.03 | 5.69 ± 1.25 |
| 5-HT$_{1B}$ | >1000 | >1000 | >1000 |
| 5-HT$_{1C}$ | >1000 | >1000 | >1000 |
| 5-HT$_{1D}$ | 277 ± 75 | 58 ± 15 | >1000 |
| 5-HT$_2$ | >1000 | >1000 | >1000 |
| 5-HT$_3$ | >1000 | >1000 | >1000 |
| GABA-A | >1000 | >1000 | >1000 |
| Benzodiazepine | >1000 | >1000 | >1000 |
| $D_1$ | >1000 | 800 ± 300 | >1000 |
| $D_2$ | >1000 | >1000 | >1000 |
| ACh-M | >1000 | >1000 | >1000 |
| $H_1$ | >1000 | >1000 | >1000 |
| Alpha$_1$ adrenergic | >1000 | >1000 | >1000 |
| Alpha$_2$ adrenergic | >1000 | >1000 | >1000 |
| Beta adrenergic | >1000 | >1000 | >1000 |

mating performance, a criterion of reversibility of behavioral response with subsequent vehicle treatment was used. Thus, a valid behavioral response to a drug treatment was arbitrarily set as a response that either did not change from the earlier control response or was reversed in the subsequent control test with vehicle. Statistical comparisons between the sexual responses to vehicle and drug treatments for each animal were made using the Wilcoxon paired-sample test (Zar, 1974).

**Evaluation of antiemetic response in cats.** Adult female cats of mixed strains were obtained by and housed in the Wright State University Animal Resources Facility, where they had free access to food and water, except during the time of testing. The cats were selected for these studies based on a minimum of two emetic episodes in five tests in response to a 30-min rotation (0.28 Hz, 17 rpm) on a motion device resembling a Ferris wheel (Crampton and Lucot, 1985). Single emetic response tests were conducted at intervals of at least 2 weeks to prevent habituation to the motion stimulus (Crampton and Lucot, 1991). Reflexive responses (occurrence of retching and vomiting) to motion after saline pretreatment were determined before and after the evaluation of a test compound. Subjects received subcutaneous injections of LY228729 in sterile saline to an injection volume of 0.1 mg/kg or vehicle 30 min before motion testing. The order of the testing was saline, 0.02, 0.005, 0.01, 0.0075, 0.0025 mg/kg and saline. The binomial data for retch/vomits were analyzed using Cochran's $Q$ test (Cochran, 1950) and McNemar's test for repeated measures (Marascuilo and McSweeney, 1977). Dose-response curves were analyzed by a program for pharmacological statistics (Tallarida and Murray, 1981).

**Alterations in punished/unpunished responding in rats.** Male Long-Evans rats (Charles River Breeding Laboratories) were housed singularly on a 12-hr light/dark cycle. The animals had free access to water but were kept on a restricted food diet to maintain their body weight between 325 and 375 g. The rats were trained to perform a two-component schedule (multiple variable interval, variable interval plus punishment schedule). The session began with the unpunished component, which used a variable interval 30-sec schedule (VI30s) of food presentation. This component was signaled by a steady light from both the house light and the lever light. The first response after a variable interval produced a food pellet with an audible click. The component

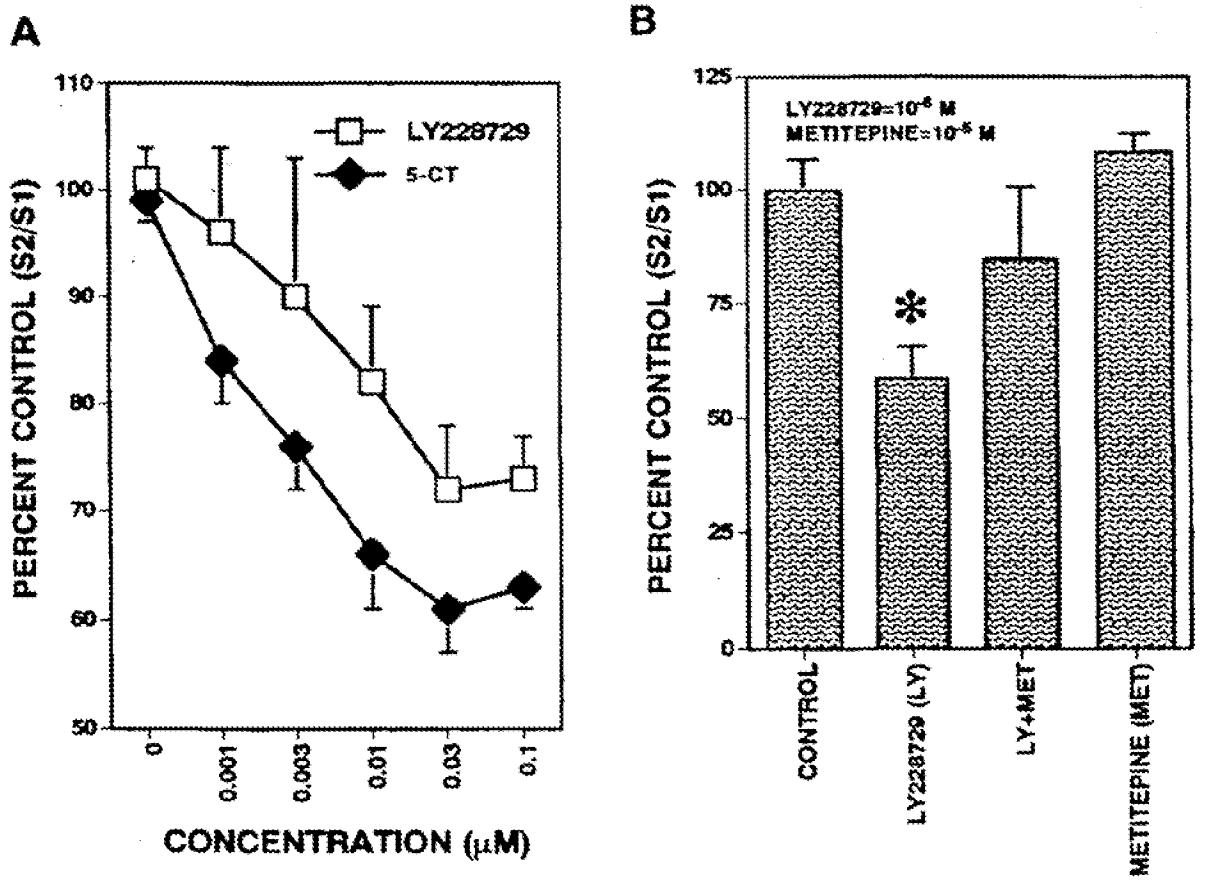


Fig. 2. Effects of LY228729 and 5-CT on $K^+$-evoked release of 5-HT from guinea pig parietal-occipital cortex slices (A) and antagonism of the effects of LY228729 ($10^{-6}$ M) with metitepine ($10^{-5}$ M) (B). Values represent the mean ± S.E.M. for five replicates and * denotes significant changes from control ($P < .05$).

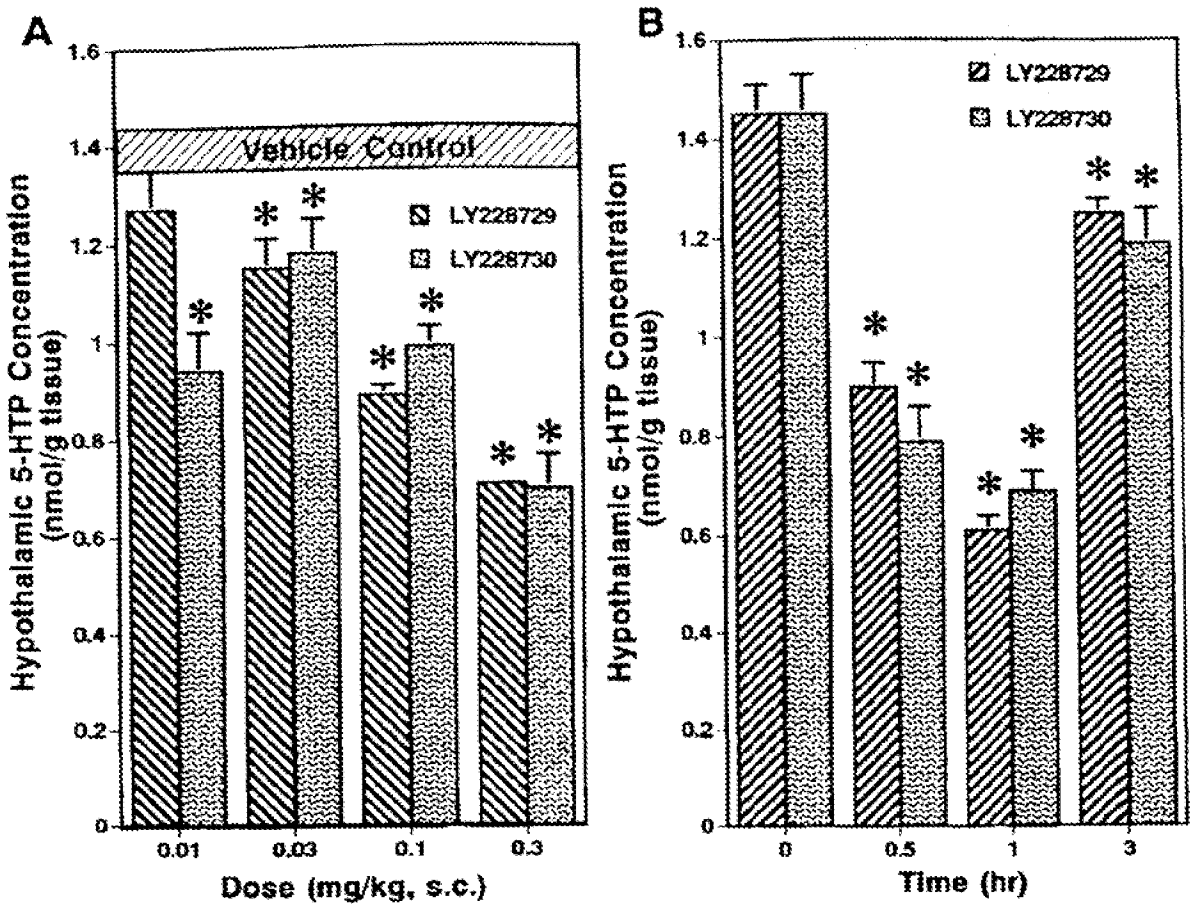


Fig. 3. Dose-dependent effects of LY228729 and LY228730 on 5-HTP accumulation in rat hypothalamic tissue after decarboxylase inhibition with NSD 1015 (A) and duration of these responses after administration of 0.3 mg/kg s.c. (B). Rats were sacrificed 40 min after NSD 1015 (100 mg/kg, i.p.) and 60 min after s.c. administration of LY228729 or LY228730. The values represent the mean ± S.E.M. for five rats per treatment group and * denotes significant changes from control ($P < .05$). Hatched area represents mean ± S.E.M. for vehicle control.

lasted for 4 min and then was followed by a 1-min blackout period during which all lights were off and responding had no consequences. The punished component followed, signaled by a steady light over the lever and a flashing house light. The punished component also used a variable interval 30-sec schedule (VI30s) of food presentation combined with a fixed-ratio 10 response (FR10) schedule of shock delivery. The shock was 0.25 mA and 0.2 sec in duration delivered through a shock scrambler to the grid floor of the test cage. Thus, in the punished component, the first response after a delay produced a food reward but every 10th response also produced a shock. This component also lasted 4 min. The unpunished and punished components alternated during the 1-hr session. These procedures are similar to those used by others for evaluation of anxiolytic activity in laboratory animals (Traber and Glaser, 1987; Barrett and Gleeson, 1991). The drug treatment schedule consisted of drug injections on Tuesdays and Fridays and vehicle (saline) injections on Thursdays. The vehicle data served as the base line for evaluating the effect of the compound on performance. The compound was administered i.p. in saline with a 20-min time out. The experiments were conducted using Skinner operant boxes (Coulbourn Instruments, E10-10, Lehigh Valley, PA), 28 × 28 × 25 cm, which were contained within light and sound attenuating shells. A 28-W house light was placed on the center line 22 cm above the grid floor. A pellet trough, 3.5 × 4 cm, was set 3 cm above the grid floor on the center line. At 8 cm to the left of the center line, a response lever was set at 3 cm and a lever light was set at 9 cm above the grid floor, respectively. All events were controlled and data were collected by a PDP-11/73 computer (Digital Equipment, Cincinnati, OH) using the SKED-11 programming language (State Systems, Kalamazoo, MI). The drug effects were evaluated for statistical differences by means of $t$ tests for paired comparison. A probability value of $P < .05$ was used for determining statistical significance.

**Evaluation of effects on punished schedule responses in pigeons.** White Carneau pigeons (450–600 g; Palmetto Pigeon Plant, Greenville, SC), maintained at 85% of their free-feeding weights, were trained to peck a Plexiglass response key mounted on the front wall of the experimental chamber. The single key was located directly above a rectangular opening through which grain could be delivered by a solenoid-operated food dispenser. The chamber was placed inside a sound- and light-attenuated enclosure that was equipped with white masking noise and a ventilating fan. The pigeons were implanted with stainless steel electrodes placed around the pubis bones (Azrin, 1959). These electrodes were connected to a plug attached to an ultrasuede jacket worn continuously by the pigeon that, during experimental sessions, was attached by a coiled cable to an electrical source that could deliver a 200-msec, 60-Hz constant-current square-wave shock. The shock intensity was adjusted for each pigeon to achieve response suppression of approximately 5% to 10% of nonpunished levels. The final shock intensity was between 2 and 5 mA for all pigeons. After the initial key-pecking training, the number of responses required to produce 3-sec access to grain was increased gradually to 30 responses (fixed ratio or FR30 schedule). Responding was maintained in two separate alternating components, each lasting 3 min. During one component, the key light was white and, during the alternate component, it was red. After responding stabilized in the two components, a schedule of shock presentation was added to the component correlated with the red key light. In this component, every 30th response produced both food and electric shock. Components continued to alternate throughout each daily session, which consisted of exposure to five components

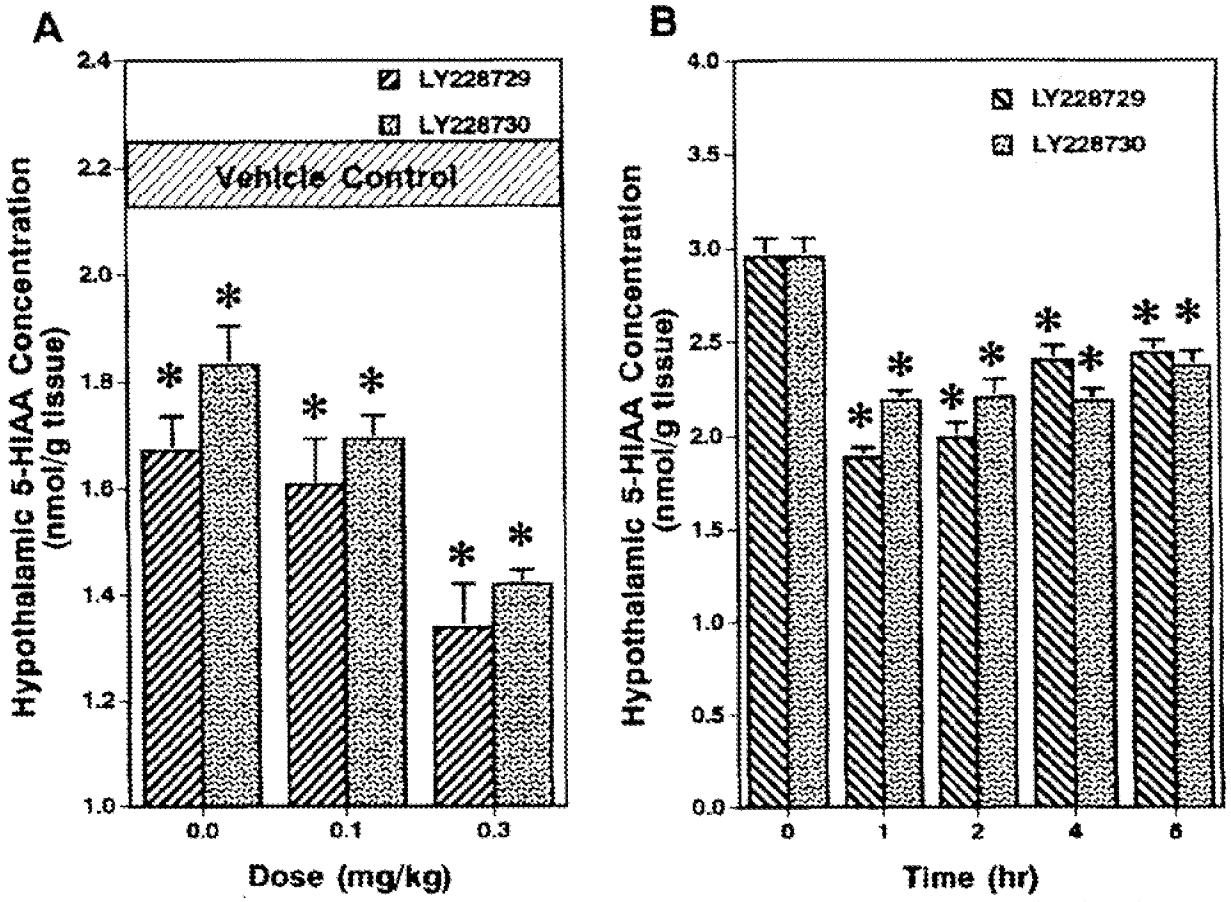


Fig. 4. Dose-dependent effects of LY228729 and LY228730 on 5-HIAA concentrations in rat hypothalamic tissue given by subcutaneous administration 1 hr before sacrifice (A) and duration of these responses after administration of 0.3 mg/kg s.c. (B). The values represent the mean ± S.E.M. for five rats per treatment group and * denotes significant changes from control ($P < .05$). Hatched area represents mean ± S.E.M. for vehicle control.

TABLE 3

**Effects of LY228729 and LY228730 on serum corticosterone concentration in rats**

Serum corticosterone values expressed as mean ± S.E.M. for five animals. All compounds were given 1 hr before sacrifice.

| Dose (mg/kg, s.c.) | Serum Corticosterone (μg/100 ml) |
|---|---|
| Control | 3.0 ± 0.54 |
| LY228729 | |
| 0.03 | 3.60 ± 0.38 |
| 0.1 | 12.96 ± 8.16 |
| 0.3 | 40.80 ± 7.08* |
| LY228730 | |
| 0.03 | 3.60 ± 0.38 |
| 0.1 | 6.40 ± 2.11 |
| 0.3 | 42.72 ± 0.97* |

* Significant difference ($P < .05$) from control group.

each of the red and white key-light colors and the correlated schedule conditions. Individual components were separated by a 30-sec period during which the chamber was dark and responding had no scheduled consequences. Drug studies were begun when responding in the two components remained stable over at least 5 days. Drugs were administered on Tuesdays and Fridays, given that responding on the previous day was within the range of control performances on the 5 days before the beginning of drug administration. LY228729 and 8-OH-DPAT were dissolved in saline with 10 μl of 0.9% lactic acid and administered into the pectoral muscle 15 min before the testing session. The results were expressed as percent of the response rate in vehicle control sessions and individual animals were averaged to derive composite dose-response curves. The drug effects were compared with the control response rates using an analysis of variance followed by a Dunnett's test. A probability value of $P < .05$ was used for determining statistical significance.

**Determination of plasma concentrations of LY228729 and metabolites in dogs, rats and rhesus monkeys.** Four female beagle dogs approximately 9 months old and weighing 7 to 10 kg were obtained from Marshall Farms (Northrose, NY). The dogs were housed in stainless steel metabolism cages. Food and water were provided *ad libitum* throughout the study. The dogs received p.o. capsules containing LY228729 hippurate dissolved in 10% acacia once daily at a dosage of 0.5 mg/kg on days 1 to 4 and 1.0 mg/kg on days 5 to 7. On day 8 of the study, the dogs received 1.0 mg/kg of $^{14}$C-LY228729 hippurate (5 μCi/kg) by gavage.

Male Sprague Dawley rats weighing 203 ± 3 g were obtained from Charles River and housed in polycarbonate metabolism cages in a light-controlled environment (12 hr light, 12 hr dark) with free access to food and water. The animals were acclimated to the housing environment for 5 days before drug administration. The rats received a 3.5 mg/kg (free base equivalents) dose of $^{14}$C-LY228729 hippurate dissolved in water (2.0 mg/rat) by oral gavage. Blood was collected from carbon dioxide-anesthetized animals by cardiac puncture using heparinized syringes (one sample/rat; three rats/time point). In all studies, blood was collected from the animals with heparinized syringes and centrifuged. The resulting plasma was stored at −70°C until the time of analysis. The concentrations reported here are presented as free base equivalents. Aliquots of plasma were diluted with Aquasure (New

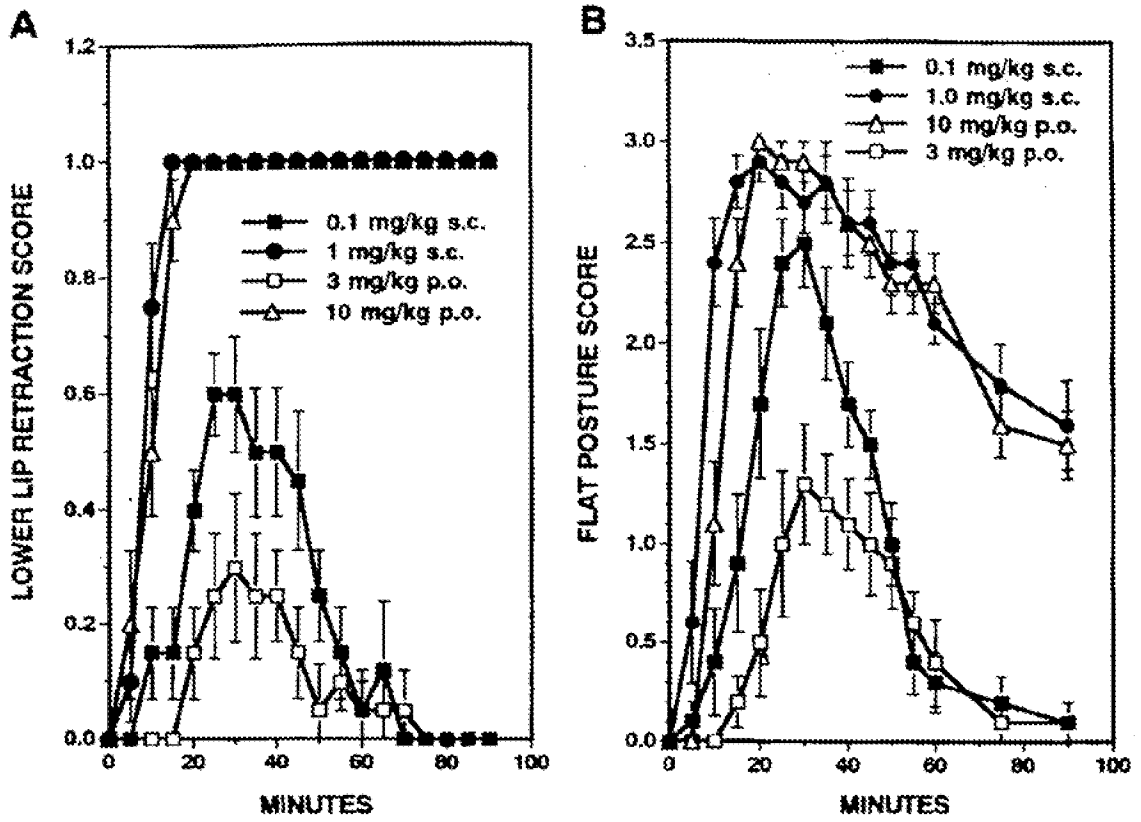


Fig. 5. Effects of LY228729 on lower lip retraction (A) and flat posture (B) responses in male rats (10 animals/dose). The lower lip retraction scores were graded on a 0 to 2 scale and the flat posture scores were graded on a 0 to 3 scale as previously described under Methods.

TABLE 4

**Comparison of 8-OH-DPAT, LY228729, LY228730 and buspirone on lower lip retraction and flat posture responses in the rat**

Values represent mean ± S.E.M. for 10 rats. The lower lip retraction response was evaluated using the 0 to 1 scale with 0 = no response, 0.5 = moderate response and 1 = maximal response (Berendsen et al., 1989). The flat posture response was evaluated using the 0 to 3 scale with 0 = no response, 1 = slight, 2 = moderate and 3 = maximal response (Smith and Peroutka, 1986).

| | Time After Treatment (min) | | | |
|---|---|---|---|---|
| | Lower Lip Retraction | | Flat Posture | |
| | 30 | 60 | 30 | 60 |
| LY228729 | | | | |
| 0.1 mg/kg s.c. | 0.60 ± 0.05* | 0.05 ± 0.05 | 2.5 ± 0.2* | 0.3 ± 0.2 |
| 1.0 mg/kg s.c. | 1.0 ± 0.0* | 1.0 ± 0.0* | 2.7 ± 0.2* | 2.1 ± 0.1* |
| 10 mg/kg p.o. | 1.0 ± 0.0* | 1.0 ± 0.0* | 2.9 ± 0.1* | 2.3 ± 0.2* |
| LY228730 | | | | |
| 1.0 mg/kg s.c. | 0.25 ± 0.09 | 0.69 ± 0.16* | 2.9 ± 0.1* | 2.9 ± 0.1* |
| 10 mg/kg p.o. | 0.95 ± 0.05* | 1.0 ± 0.0* | 2.9 ± 0.1* | 2.9 ± 0.1* |
| 8-OH-DPAT | | | | |
| 0.1 mg/kg s.c. | 0.90 ± 0.07* | 0.45 ± 0.14* | 2.5 ± 0.2* | 1.1 ± 0.2* |
| 1.0 mg/kg s.c. | 0.90 ± 0.07* | 0.80 ± 0.08* | 2.8 ± 0.1* | 1.9 ± 0.1* |
| Buspirone | | | | |
| 1.0 mg/kg s.c. | 0.95 ± 0.05* | 0.20 ± 0.08 | 2.4 ± 0.2* | 0.8 ± 0.3 |
| 10 mg/kg s.c. | 1.0 ± 0.0* | 0.95 ± 0.05* | 2.8 ± 0.1* | 2.7 ± 0.2* |

* Significant change from base-line response ($P < .05$). Inactive doses: LY228729 0.01 mg/kg s.c.; 1 mg/kg p.o.; LY228730 0.01 mg/kg s.c., 1 mg/kg p.o., 8-OH-DPAT 0.01 mg/kg s.c.; 10 mg/kg p.o., buspirone 0.1 mg/kg s.c., 10 mg/kg p.o.

TABLE 5

**Antagonism of lower lip retraction and flat posture responses to LY228729 (0.3 mg/kg s.c.) with (±) pindolol (10 mg/kg s.c.)**

Responses were evaluated as in Table 4. Values represent mean ± S.E.M. for 10 rats at 30 min after treatment with LY228729. Pindolol was given 15 min before LY228729.

| | Time After Treatment | |
|---|---|---|
| | Lower Lip Retraction | Flat Posture |
| Vehicle + LY228729 | 1.0 ± 0.0 | 3.0 ± 0.0 |
| Pindolol + Vehicle | 0.0 ± 0.0* | 0.0 ± 0.0* |
| Pindolol + LY228729 | 0.1 ± 0.1* | 1.0 ± 0.2* |

* Significant difference from LY228729 response ($P < .05$).

England Nuclear, Wilmington, DE) and the radioactivity was estimated by liquid scintillation spectrometry. All samples were corrected for quench and counting efficiency by standard external calibration.

Three young adult, female rhesus monkeys weighing 3.8 to 6.4 kg were obtained from stock animals at the Lilly Research Laboratories (Indianapolis, IN) monkey colony. The animals were individually housed in stainless steel metabolism cages with free access to food and water. The monkeys received a single 10 mg/kg (20 μCi/kg, 1 ml/kg) dose of $^{14}$C-LY228729 hippurate dissolved in a 10% acacia vehicle by oral gavage.

Plasma samples were extracted and analyzed by reverse-phase high-performance liquid chromatography with a Perkin Elmer (Beaconsfield, England) LS40 fluorescence detector, which was operated under the total emission mode with an excitation wavelength of 240 nm and a 390-nm emission filter. Fifty microliters of a solution containing (−)-4-dipropylamino-1,3,4,5-tetrahydro-4-propylaminobenz[c,d]indole-6-carbonitrile as an internal standard and 0.5 ml of phosphate buffer (0.025 M, pH 7) were added to a plasma aliquots (0.25–0.5 ml). The contents were mixed and added to 100 mg of solid-phase cyano extraction cartridges (Analytichem International, Harbor City, CA) precon-

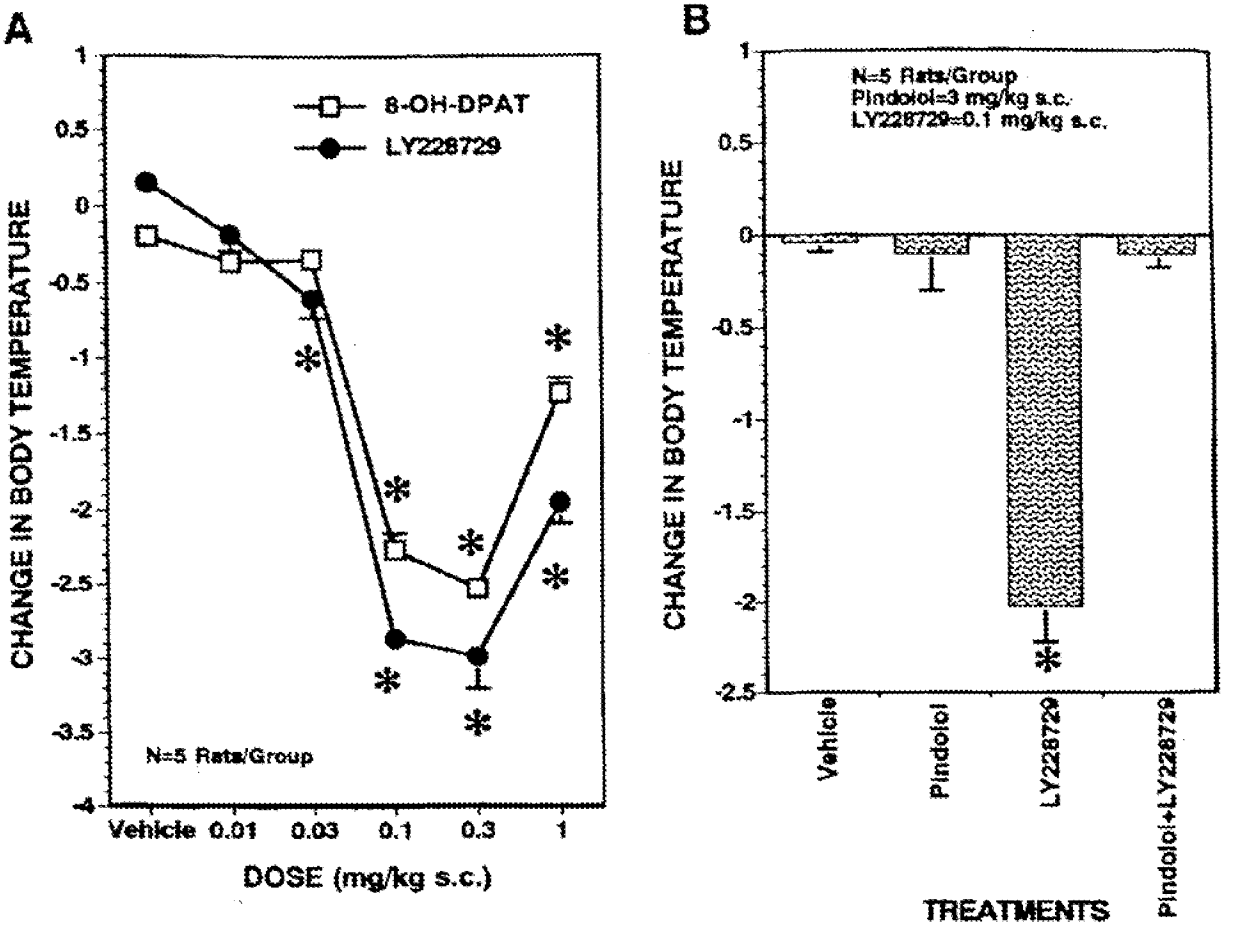


Fig. 6. Dose-dependent effects of LY228729 on body temperature in rats (A) and antagonism of the responses to LY228729 (0.1 mg/kg s.c.) with pindolol (3 mg/kg s.c. 15 min before LY228729) (B). Measurements were taken immediately before LY228729 and 30 min later. The values represent the mean ± S.E.M. of five rats/group for the change in body temperature (in degree Celsius) and * denotes significant changes from control ($P < .05$).

ditioned with a 1-column volume each of methanol, elution solvent (30-40-30; methanol-25 mM ammonium phosphate buffer (pH 5)-acetonitrile), water and buffer. The cartridges were washed with 1 ml each of water and 50 mM ammonium phosphate buffer, pH 5. The drugs were then eluted from the cartridges directly into autosampler vials with 0.5 ml of elution solvent. Aliquots were analyzed by high-performance liquid chromatography with a 4.6 × 250 mm Apex I cyanopropyl column (5 μm, Jones Chromatography, Lakewood, CO) under isocratic conditions. The mobile phase consisted of acetonitrile-methanol-ammonium phosphate (30-30-40) and the flow rate was 1 ml/min. The retention times for LY228729, LY289211 (despropyl-LY228729) and the internal standard were 450, 385 and 680 sec, respectively.

## Results

**Effects of LY228729 and LY228730 on radioligand binding to receptors in rat brain membrane preparations.** LY228729 and LY228730, its opposite (+) enantiomer, exhibited high selectivity and affinity for the 5-HT$_{1A}$ receptor with $K_i$ values of 0.13 ± 0.01 nM and 0.28 ± 0.03 nM for the displacement of $^3$H-8-OH-DPAT (table 2). There was no significant difference between the $K_i$s for the 5-HT$_{1A}$ receptor for these enantiomers (Student's $t$ test). In addition to the binding at the 5-HT$_{1A}$ receptor, both compounds also had weak affinity for the 5-HT$_{1B}$ receptor (table 2). The despropyl metabolite of LY228729, LY289211, had a similar selectivity but a statistically lower affinity for the 5-HT$_{1A}$ receptor than LY228729 and LY228730 (analysis of variance-Dunnet's test; table 2).

**Effects of LY228729 and LY228730 on K$^+$-evoked $^3$H-serotonin release from slices of guinea pig parietal-occipital cortex.** LY228729 induced a concentration-dependent reduction of stimulus-evoked release of 5-HT, which reached statistical significance at $3 \times 10^{-7}$ M, which was the approximate $EC_{50}$ (fig. 2A). The maximal suppression of 5-HT release was 40% with 5-CT and 25% with LY228729. The effect of $10^{-6}$ M LY228729 was blocked with $10^{-5}$ M metitepine (fig. 2B). LY228730 and 8-OH-DPAT did not affect the release of 5-HT in concentrations as high as $10^{-5}$ M (data not shown).

**Effects of LY228729 and LY228730 on concentrations of monoamines and their metabolites in rat brains.** In rats treated with NSD 1015 (100 mg/kg i.p.) 30 min before sacrifice, LY228729 (0.03–0.3 mg/kg s.c.) and LY228730 (0.01–0.3 mg/kg s.c.) produced reductions in hypothalamic concentrations of 5-HTP (fig. 3A). At a dose of 0.3 mg/kg s.c. LY228729 reduced hypothalamic concentrations of 5-HTP for 3 hr compared with 5 hr for LY228730 (fig. 3B). Both compounds (0.03–0.3 mg/kg s.c.) produced significant reductions in hypothalamic concentrations of 5-HIAA (fig. 4A). At a dose of 0.3 mg/kg s.c. both compounds reduced hypothalamic concentrations of 5-HIAA for at least 6 hr (fig. 4B). The despropyl metabolite of LY228729, LY289211, did not alter 5-HIAA concentrations at doses of 0.3, 1 and 3 mg/kg s.c. (data not shown).

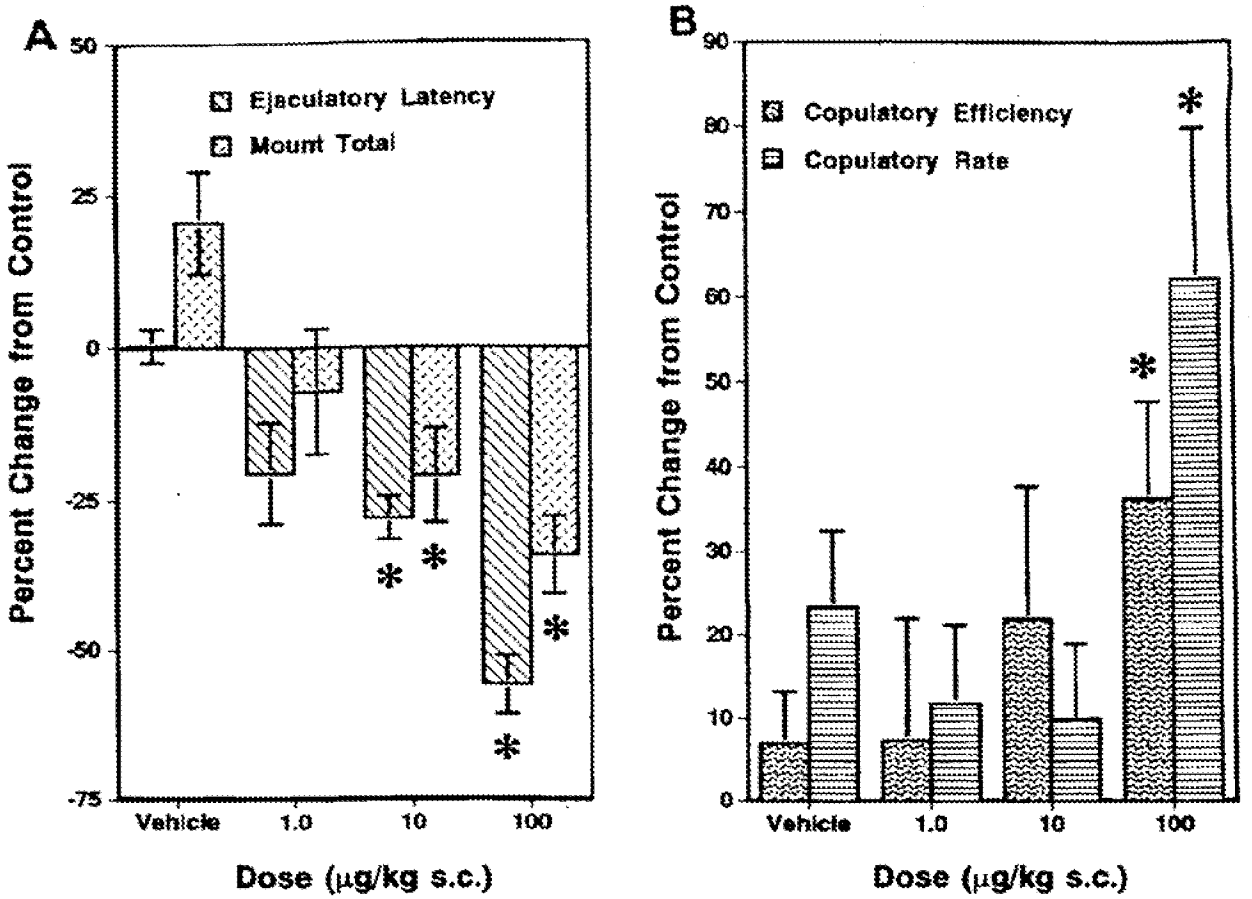


Fig. 7. Effects of LY228729 on ejaculatory latency and mount total (A) and copulatory efficiency and copulatory rate (B) of male rats. Values represent the mean ± S.E.M. for the change in each parameter from the control response for the same animal after a vehicle treatment during an earlier test. The minimal number of rats used for these groups was 10 and * denotes significant changes from control ($P < .05$).

TABLE 6

**The suppression of motion sickness in the cat by LY228729**

Compounds were given 0.5 hr before testing.

| Treatment | # Vomiting/# Tested |
|---|---|
| Saline | 7/13 |
| LY228729 (mg/kg s.c.) | |
| 0.0025 | 6/13 |
| 0.005 | 5/13 |
| 0.0075 | 1/13* |
| 0.010 | 0/11* |
| 0.02 | 0/11* |
| Saline | 13/13 |

* Significant difference ($P < .05$) from control group.

**Effects of LY228729 and LY228730 on serum corticosterone concentrations.** LY228729 and LY228730 induced dose-related increases in serum corticosterone concentrations at 1 hr after s.c. administration. These changes reached statistical significance at the 0.3-mg/kg dose (table 3). LY289211, did not alter serum corticosterone concentrations at doses of 0.3, 1 and 3 mg/kg s.c. (data not shown).

**Effects of LY228729 and LY228730 on 5-$HT_{1A}$ receptor mediated behaviors in the rat.** LY228729 induced significant and dose-related increases in flat posture and lower lip retraction responses (fig. 5). In addition to these responses, LY228729 also produced forepaw treading and head weaving at 1 mg/kg s.c. and 10 mg/kg p.o. After subcutaneous administration, LY228729 was approximately equipotent to 8-OH-DPAT and more potent than LY228730 and buspirone in both of these behaviors (table 4). However, LY228729 produced maximal behavioral responses after a dose of 10 mg/kg p.o., whereas 8-OH-DPAT and buspirone were inactive at this p.o. dose (table 4). LY289211, the major identified metabolite, was inactive at doses of 1 mg/kg s.c. (data not shown). The effects of 0.3 mg/kg s.c. LY228729 were blocked by pretreatment (15 min before LY228729) with 10 mg/kg s.c. (±) pindolol (table 5).

**Effects on body temperature in male rats.** LY228729 induced dose-dependent decreases in body temperature in rats, which reached statistical significance doses of 0.03 to 1 mg/kg s.c. (fig. 6A). LY228729 was slightly more potent than 8-OH-DPAT in these experiments. The effects of LY228729 (0.1 mg/kg s.c.) were blocked by pretreatment with (±) pindolol (3 mg/kg s.c.) 15 min before LY228729 (fig. 6B).

**Effects of LY228729 on male rat sexual behavior.** LY228729 induced dose-related decreases in ejaculatory latency and the total number of mounts required for ejaculation (fig. 7A). These effects were statistically significant at doses of 10 and 100 μg/kg s.c. LY228729 also induced statistically significant increases in copulatory efficiency and copulatory rate at 100 μg/kg s.c. (fig. 7B). Other standard copulatory performance indices, such as mount, intromission and postejaculatory mount latencies and intromission frequency, were not significantly altered by these doses. By contrast with these augmenting effects on sexual behavior, all rats tested ($n = 7$) at 1 mg/kg

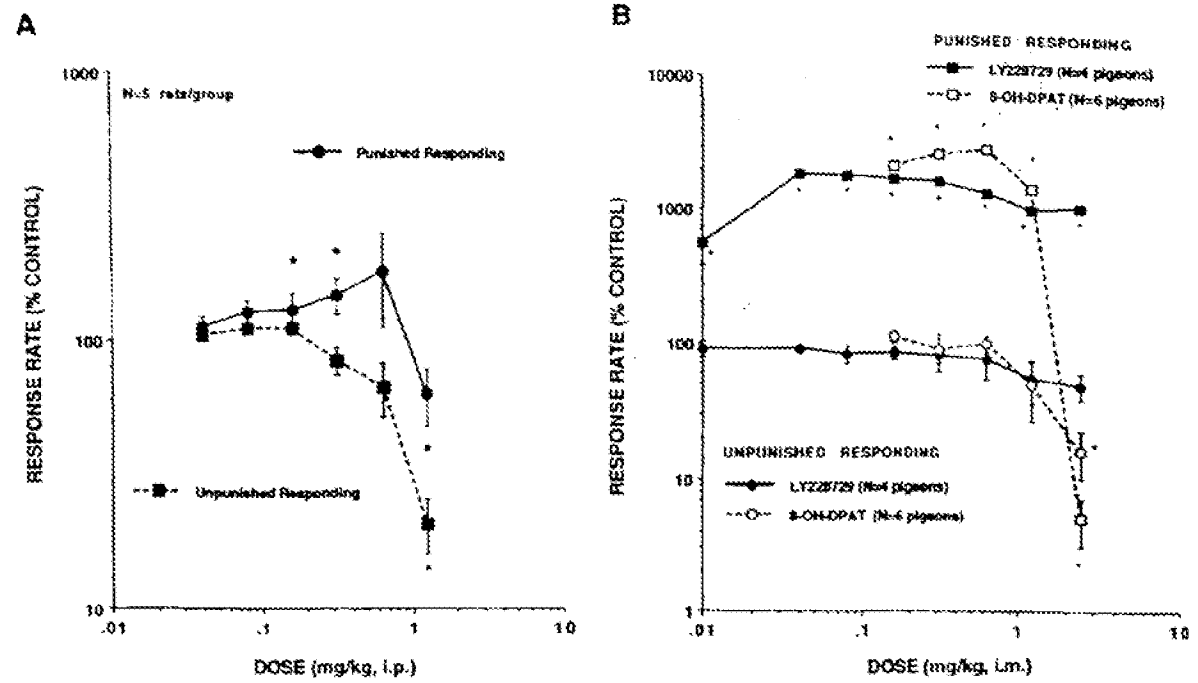


Fig. 8. Effects of LY228729 on punished and unpunished responding in male rats (A). Circles and squares represent mean ± S.E.M. (five rats/point) punished responding responses and unpunished responding responses, respectively. Effects of LY228729 (circles) and 8-OH-DPAT (squares) on punished (solid symbols) and unpunished responding (open symbols) in pigeons (B). Each point represents the mean ± S.E.M. for effects determined in six pigeons for 8-OH-DPAT and four pigeons for LY228729. Mean control response rates were 2.00 ± 0.64 (unpunished) and 0.05 ± 0.03 (punished) for LY228729 and 2.22 ± 0.61 (unpunished) and 0.03 ± 0.03 (punished) for 8-OH-DPAT. The * denotes significant changes from control ($P < .05$).

s.c. failed to mount during the 30-min test period. At this dose, these rats also exhibited maximal 5-HT$_{1A}$ syndrome responses, including flat posture, lower lip retraction, forepaw treading and head weaving. These latter stereotyped behaviors may have suppressed the sexual performance by interfering with the ability of the animal to focus on the goal-directed sexual behaviors.

**Effects of LY228729 on emetic response in cats induced by movement.** LY228729 significantly suppressed motion sickness in cats at doses of 0.0075 to 0.2 mg/kg s.c. (table 6; $Q = 25.06$, 4 *df*, $P < .001$). Significantly more emetic responses were observed in the control test performed after evaluation of LY228729 compared with the control test before drug evaluation ($P = .03$). This latter observation is considered to be a random event, which occasionally occurs with this type of experimentation. However, the difference between the control groups makes it necessary to express the descriptive analysis of the data as a range. The $ED_{50}$ for LY228729 was between 0.0029 and 0.005 mg/kg (probit analysis). At the doses tested, LY228729 did not produce any change in behavior. This is in contrast to the effects of buspirone, which elicits strong defensive behavior in cats at doses that produce submaximal suppression of emetic responses (Lucot and Crampton, 1987).

**Effects of LY228729 on punished/unpunished responding in rats.** LY228729 produced dose-related increases in the punished responding rates and in higher doses dose-related decreases in unpunished responding rates (fig. 8A). The increases in punished responding reached statistical significance at doses of 0.08 and 0.32 mg/kg i.p. for LY228729. The decreases in unpunished responding reached statistical significance at 0.64 and 1.25 mg/kg i.p. for LY228729.

**Effects on punished schedule responses in pigeons.** Vehicle control performance in birds treated with either LY228729 or 8-OH-DPAT was similar. Unpunished responding was characterized by brief pauses after food delivery followed by high response rates; punished responding was characterized by relatively few responses during the component. LY228729 produced significant increases in punished responding in all four animals across a wide range of doses from 0.04 to 2.5 mg/kg (fig. 8B). The two highest doses tested decreased unpunished responding but did not reduce punished responding below that seen at lower doses. The peak dose (0.04 mg/kg) increased punished response rates 1800% over the control rate. 8-OH-DPAT produced effects on punished and unpunished responding that were comparable to those seen with LY228729. As with LY228729, 8-OH-DPAT produced significant increases in punished responding at doses that did not affect unpunished behavior. However, at the highest dose tested (2.5 mg/kg), 8-OH-DPAT almost completely eliminated both punished and unpunished responding in all birds, an effect not seen with LY228729.

**Plasma concentrations of LY228729 and metabolites in dogs, rats and rhesus monkeys.** The plasma concentrations of LY228729 as determined by high-performance liquid chromatography-fluorescence detection for Fischer 344 rats, rhesus monkeys and beagle dogs are shown in figure 9. The rhesus monkeys and the Fischer 344 rats achieved maximal plasma concentrations at approximately 2 hr, whereas the beagle dogs achieved maximal plasma concentrations at approximately 1 hr. The dogs also had a more rapid clearance after the peak plasma concentrations compared with the rats and monkeys. The pharmacokinetic parameters of parent drug and its despropyl metabolite in Fischer 344 rats, rhesus monkeys and beagle dogs as determined by chromatographic analysis of $^{14}$C-LY228729 are shown in figures 10 and table 7. In the Fischer 344 rats and rhesus monkeys, the despropyl metabolite

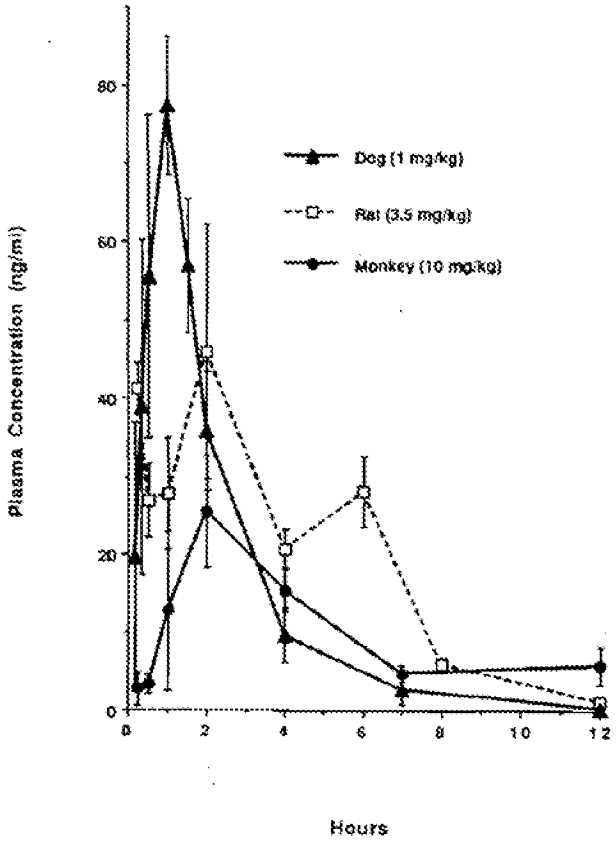


Fig. 9. Plasma concentrations of LY228729 after oral administration to rats, monkeys and dogs as determined by high-performance liquid chromatography with fluorescence detection.

achieved higher plasma concentrations and had a slower clearance compared with the parent compound (figs. 10AB and table 7). In the dogs, this pattern was reversed with the parent compound representing the major identified fraction of radioactivity (fig. 10C, table 7). However, the information from the dogs was obtained from a multidose study rather than a single-dose study and the apparent species variance could be complicated by this dosing variance. In all animal species, a substantial portion of the total radioactivity was not identified.

## Discussion

The results of the *in vitro* radioligand displacement studies indicate that LY228729 and its opposite enantiomer, LY228730, have a high affinity and selectivity for the 5-$HT_{1A}$ receptor. Both compounds also had weaker affinities for 5-$HT_{1D}$ receptor and were relatively inactive ($K_i$s greater than 1 $\mu$M) in other receptor assays. The only differences between these enantiomers were that LY228729 had a lower $K_i$ for the 5-$HT_{1A}$ receptor and higher $K_i$ for the 5-$HT_{1D}$ receptor but neither of these differences was statistically significant. The despropyl metabolite of LY228729, LY289211, had a similar selectivity to the parent compound but a significantly lower affinity for the 5-$HT_{1A}$ receptor. Therefore, both propyl groups were required for maximal activity in these *in vitro* assays.

LY228729, but not LY228730 or 8-OH-DPAT, induced dose-related suppression of $K^+$-evoked release of $^3$H-5HT from slices of guinea pig parietal occipital cortex. These effects were antagonized by metitepine, which is a known antagonist for the terminal 5-HT autoreceptor (Limberger *et al.*, 1991). In these experiments, LY228729 was less potent and produced a smaller maximal suppression than did 5-CT. This latter observation suggested that LY228729 is a partial agonist for the synaptic terminal autoreceptors, which have been previously classified as 5-$HT_{1D}$ receptors (Hoyer *et al.*, 1990; Limberger *et al.*, 1991). The higher binding affinity of LY228730 compared with that of LY228729 in the 5-$HT_{1D}$ receptor binding assay may not be indicative of the intrinsic activity of these compounds because LY228729 was more potent in the suppression of 5-HT release than was LY228730. However, the effects on 5-HT release may be related to a 5-$HT_{1D}$ receptor subtype, which is not adequately characterized by current standard displacement assays (Weinshank *et al.*, 1992) or to species and tissue differences between these assays.

The 5-$HT_{1A}$ receptor agonists are known to suppress the activity of serotonergic neurons through the activation of autoreceptors on the cell bodies of these neurons (Sprouse and Aghajanian, 1986; Hjorth and Magnusson, 1988; Larsson *et al.*, 1990). Biochemical correlates of the inhibition of 5-HT synthesis and release include reductions in 5-HTP concentrations after decarboxylase inhibition and a lowering of 5-HIAA concentrations, respectively (Hjorth and Magnusson, 1988). LY228729 and its opposite enantiomer elicited both of these effects at doses of 0.03 mg/kg s.c. For comparison, the minimal effective dose of buspirone to produce significant reductions in 5-HIAA levels and 5-HTP accumulation was previously determined to be 1 mg/kg s.c. (Fuller and Perry, 1989).

The serotonergic control of the hypothalamic-pituitary-adrenal axis is currently believed to be mediated by 5-$HT_2$, 5-$HT_{1C}$, and 5-$HT_{1A}$ receptors (Fuller, 1990). These receptors induce the release of corticotropin releasing hormone. This stimulates the secretion of adrenocorticotropic hormone, which in turn, stimulates the secretion of glucocorticoids (Calogero *et al.*, 1989; Gilbert *et al.*, 1988; King *et al.*, 1989; Fuller, 1990). Both LY228729 and its opposite enantiomer, LY228730, induced similar dose-related increases in plasma corticosterone concentrations with statistically significant changes occurring at a dose of 0.3 mg/kg s.c. In previous reports, 8-OH-DPAT induced significant increases in plasma corticosterone concentrations at 0.1 mg/kg s.c. (Koenig *et al.*, 1987; Fuller and Snoddy, 1990), whereas buspirone, ipsapirone and gepirone induced increases at 3 mg/kg s.c. (Koenig *et al.*, 1988).

In rats, 5-$HT_{1A}$ receptor agonists induce a variety of characteristic responses, including a variety of behavioral responses, such as lower lip retraction, flat posture, reciprocal forepaw treading, hindlimb abduction, side to side head weaving, straub tail, increased locomotor activity and hyperreactivity (Berendsen *et al.*, 1989; Smith and Peroutka, 1986) and reductions in body temperature (Hjorth, 1985). In the present studies, lower lip retraction and flat posture were chosen as behavioral indices because they were the most sensitive behavioral responses. LY228729 induced increases in both flat posture and lower lip retraction at doses equal to or greater than 0.1 mg/kg s.c. and 3 mg/kg p.o. LY228729 was equipotent to 8-OH-DPAT and more potent than buspirone by subcutaneous administration but it was more potent than either compound by oral administration. Both of these behavioral responses were prevented by pretreatment with 10 mg/kg (±) pindolol, a 5-$HT_{1A}$ receptor antagonist, confirming that these responses were a result of stimulation of the 5-$HT_{1A}$ receptor. LY228729 was also shown to lower body temperature with a similar profile to that of 8-OH-DPAT, a selective 5-$HT_{1A}$ receptor agonist, and these responses were blocked by (±) pindolol. These studies indicate that LY228729 is an orally active agonist for the 5-$HT_{1A}$ receptor.

General pharmacology studies have established that LY228729 is a potent 5-$HT_{1A}$ agonist that is orally active and has a duration of action suitable for clinical evaluation. These

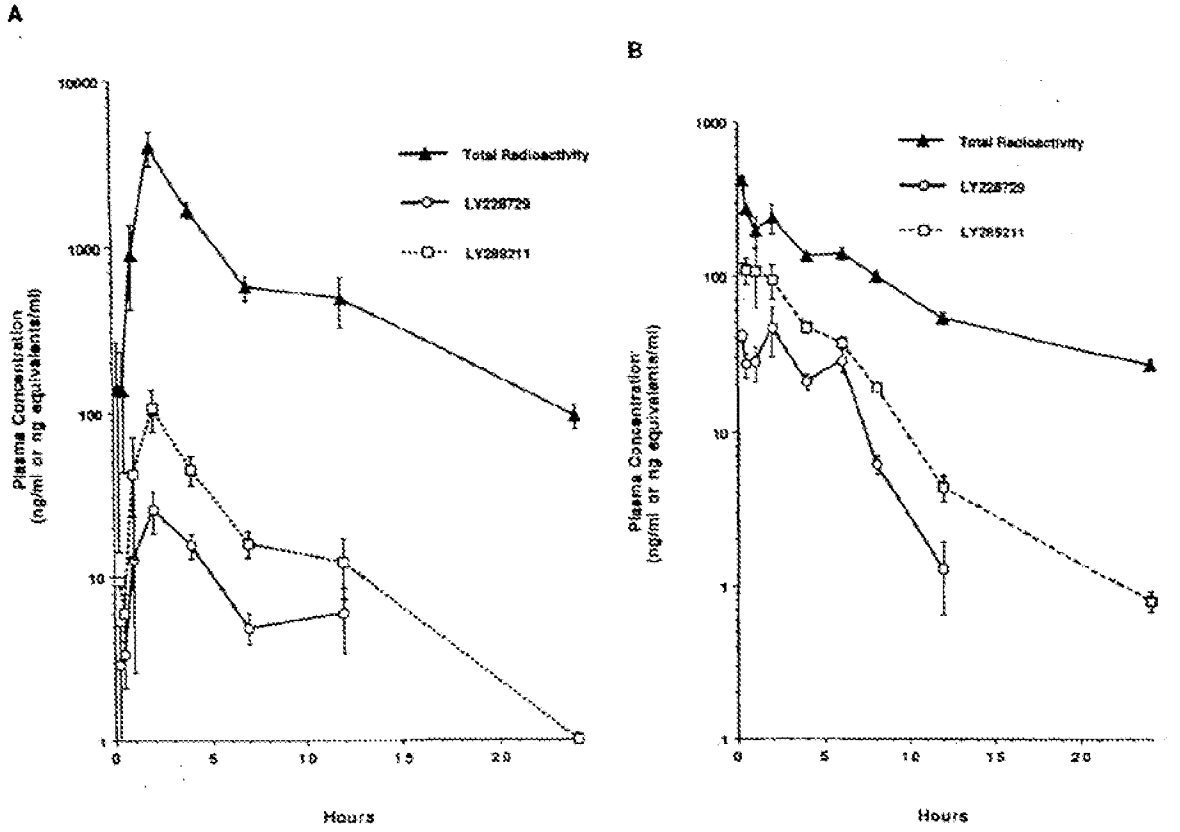


Fig. 10. Plasma concentrations of $^{14}$C-LY228729, $^{14}$C-LY289211 and total radioactivity in rats (A), monkeys (B) and dogs (C) after administration of $^{14}$C-LY228729.

TABLE 7

**Pharmacokinetic parameters of LY228729 and its despropyl metabolite, LY289211, in monkeys, rats and dogs after oral administration**

| Species | Dose (mg/kg) | $C_{max}$ (ng/ml) | $T_{max}$ (hr) | Area Under the Curve (ng-hr/ml) | $T_{1/2}$ (hr) |
|---|---|---|---|---|---|
| Monkey | | | | | |
| LY228729 | 10 | 26 | 2 | 122 | 2.3 |
| LY289211 | | 107 | 2 | 476 | 3.5 |
| Rat | | | | | |
| LY228729 | 3.5 | 46 | 2 | 225 | 1.9 |
| LY289211 | | 113 | 0.25 | 540 | 3.2 |
| Dog | | | | | |
| LY228729 | 1.0* | 89 | 0.7 | 173 | 1.2 |
| LY289211 | | 4.5 | 0.7 | 4 | — |

* Multiple dose study. Dogs received daily doses of 0.5 mg/kg on days 1 to 4 and 1 mg/kg on days 4 to 7. On day 8, dogs received 1 mg/kg of $^{14}$C-LY228729. Kinetic data were calculated from day 8. Formula weights for LY228729 and LY289211 were 299.4 and 257.3 g/mol, respectively.

conclusions were further supported by the drug disposition studies in dogs, rats and monkeys, which have shown that LY228729 is rapidly absorbed after oral administration and is detectable in plasma 4 to 6 hr after dosing. The major metabolic route for LY228729 in the species evaluated is through the removal of one propyl group on the side chain amide. This results in a 50-fold reduction in affinity for the 5-HT$_{1A}$ binding affinity *in vitro* and no significant activity in *in vivo* assays, such as neurochemical estimates of 5-HT activity, elevations in serum corticosterone concentrations and 5-HT syndrome behaviors.

In addition to the pharmacological assays that were used to define the receptor selectivity and potency, LY228729 was also evaluated in animal models using indices of emetic response in cats, operant behavior in pigeons and sexual response in rats. These models are thought to be predictive of efficacy in the clinical disorders of motion sickness, anxiety and sexual dysfunction (Lucot and Crampton, 1989; Barrett and Gleeson, 1991; Foreman and Wernicke, 1990).

The 5-HT$_{1A}$ agonists, such as 8-OH-DPAT, buspirone and flesinoxan, have been previously shown to suppress emetic response to provocative motion and various chemical stimuli (Lucot, 1991; Lucot and Crampton, 1987; 1989). In the current studies, LY228729 suppressed the emetic response to provocative motion with an approximate $ED_{50}$ of 0.006 mg/kg s.c. LY228729 was 5 to 7.2 times more potent than 8-OH-DPAT and 30 times more potent than buspirone in blocking the emetic responses to motion (Lucot and Crampton, 1987; 1989). These findings provide further evidence of 5-HT$_{1A}$ receptor agonism and suggest a possible clinical utility for this compound as an antiemetic.

In a model for sexual response in male rats, LY228729 decreased ejaculatory latency and the number of mounts required for ejaculation. These findings indicate the LY228729 can lower the latency and stimulus requirements for this sexual reflex. LY228729 also induced increases in the copulatory efficiency and rate. The increased copulatory efficiency (number of intromissions/total number of mounts) suggests that LY228729 improved the capacity of these rats to achieve erections sufficient for intromission. The increased copulatory rate indicates that LY228729 elevated sexual drive. These findings

also suggest that LY228729 would be useful for the disorders related to central nervous system regulation of erectile response, sexual drive and orgasmic reflexes. These proposed clinical utilities are in agreement with previous observation of increases sexual responses in patients after treatment with the 5-$HT_{1A}$ receptor agonist, buspirone (Othmer and Othmer, 1987).

The most widely accepted preclinical tests for anxiolytic activity are the punished responding or conflict tests, which have been previously used to characterize the activity of buspirone, gepirone and ipsapirone (Traber and Glaser, 1987; Barrett and Gleeson, 1991; Chojnacka-Wojcik and Przegalinski, 1991). In the conflict tests with rats, LY228729 significantly increased punished responding at doses that did not affect unpunished responding. In the conflict tests in pigeons, LY228729 produced increases in punished responding at all doses tested (0.4–2.5 mg/kg i.m.) without altering unpunished responding. These findings indicate that LY228729 has the preclinical characteristics of a compound with anxiolytic activity. In addition to the preclinical findings, the clinical studies with the 5-$HT_{1A}$ partial agonists, buspirone (Feighner, 1987; Jann, 1988; Petracca *et al.*, 1990), gepirone (Harto *et al.*, 1988) and ipsapirone (Borison *et al.*, 1990) also justify this indication.

The clinical evaluation of LY228729 for efficacy in treating mental depression is also justified because buspirone (Fabre, 1990; Rickels *et al.*, 1991), gepirone (Jenkins *et al.*, 1990; Amsterdam, 1992) and ipsapirone (Heller *et al.*, 1990) have been found to be efficacious in this disorder. Although no preclinical data supporting this indication were presented in this summary of preclinical studies, LY228729 has recently been found to have potent antidepressant-like activity in the forced swim model in rats (Benvenga and Leander, submitted for publication). Buspirone, gepirone, ipsapirone and 8-OH-DPAT are also active in this model (Wieland and Lucki, 1990).

In summary, the preceding preclinical studies have demonstrated that LY228729 is a potent and selective 5-$HT_{1A}$ agonist with sufficient oral activity and duration of action for clinical evaluation. The preclinical studies that were used to characterize the general pharmacological effects of LY228729 in rats found parenteral activity in the range of 0.03 to 0.3 mg/kg s.c. and oral activity in the range of 3 to 10 mg/kg. The duration of effect on brain neurochemistry was 3 to 6 hr at a dose of 0.3 mg/kg s.c. In the models of preclinical efficacy, LY228729 enhanced sexual performance of male rats in the dose range of 0.01 to 0.1 mg/kg s.c. and suppressed the emetic response of cats induced by motion in a dose range of 0.0075 to 0.2 mg/kg s.c. the anxiolytic-like activity in rats was observed in the dose range of 0.08 to 0.32 mg/kg i.p. and the anxiolytic activity in pigeons was found in a dose range of 0.4 to 2.5 mg/kg i.m. These studies provided preclinical justification for the use of LY228729 in the treatment of sexual response disorders, motion sickness and anxiety. Additional preclinical studies are planned for other possible utilities, such as depression and eating disorders.

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